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EFFECTS OF SODIUM TETRAMETAPHOSPHATE ON THE CASEIN COLLOID IN MILK

T. F. FORD, W. L. CHOATE and F. A. HECKMAN
Eastern Utilization Research Branch
Agricultural Research Service - U.S.D.A., Washington 25, D. C.

The casein-containing colloidal aggregates in skim milk can be reduced in size and translucent liquids obtained in a number of ways; by treatment with ion exchange resins, by adding certain detergents, and by adding sodium tetrametaphosphate. The last two treatments have been found to give very few different sizes. The possibility of dispersion by such relatively mild treatments is of interest in respect to the original state of casein in milk. The phosphates are of great biological importance. A study of the sodium tetrametaphosphate-skim milk system is here reported.

Experimental methods

Preparation of solutions. All of the milks used were fresh morning's milk from a single Jersey cow. The whole milks were separated 3 to 4 hours after milking, and the skim milks stored at 4°C. Nothing was added at this stage. Twenty-four hours after separating, each sample of skim milk was diluted with an equal volume of buffer, and sodium tetrametaphosphate then added in the amount required to make the solution 0.28 M in this salt, a concentration of sodium tetrametaphosphate somewhat in excess of the minimum required for producing translucent solutions. The casein concentration was thus lowered to about 1.5%.

Preliminary experiments disclosed an appreciable increase in viscosity of the samples in the first several minutes after treatment, followed by a rapid decrease of about the same magnitude, and a gradual approach to equilibium during several hours. Accordingly, solutions were allowed to stand 24 hours at room temperature (20°C) before ultracentrifugal and viscosity measurements were made. No effects due to bacterial action were observed even

after several days at room temperature. All data reported are on samples prepared and aged as described.

Experiments were conducted in series, samples being prepared from fresh milk on each of the first three days of the week, and these solutions then analyzed in the ultracentrifuge 48 hours after separating the milk, that is, on the last three days of the week. In each series the pH was varied in a progressive manner. By this procedure, and by using milk from a single animal, it was hoped that effects of differences in milk composition would be minimized.

A universal buffer containing sodium barbital, sodium chloride, sodium acetate, and hydrochloric acid with a final ionic strength of 0.20 was used in diluting the skim milk samples. Small amounts of sodium hydroxide were sometimes added to raise the pH. Each batch of sodium tetrametaphosphate was examined in the petrographic microscope and by X-ray powder diffraction. The petrographic examination revealed that in each sample essentially only one phase was present. The X-ray diffraction patterns indicated that the crystal structures of the three samples were identical. The patterns were in good agreement with published data (2) for the high-temperature form of sodium tetrametaphosphate tetrahydrate. Thus, both the identity and purity of the reagent were satisfactorily established.

Physical measurements. For optical ultracentrifugal measurements, the Spinco Model E ultracentrifuge and a standard 12 mm. cell were used. In sedimentation velocity experiments a rotor speed of 50,740 r.p.m. was used and pictures were taken at 16 or 32 minute intervals. True rotor temperatures were taken as the midpoints between intervals on a linear plot of temperature against time, the initial temperature being assumed at the time at which the rotor reached speed and the final temperature being assumed at the time at which the rotor brake was applied. An acceleration temperature drop of 0.65°C was taken for this speed from the data of Waugh and Yphantis (7). In sedimentation equilibrium experiments, speeds of 6,944 and 11,192 r.p.m. were used, and an equilibrium temperature approaching the final rotor temperature assumed.

The Spinco separation cell was used for fractionation. An air-driven bowl rotor with an annular partition was used for the same purpose and for progressive depletions of solutions. Details

of construction and use of this bowl rotor will be described elsewhere.

Both constant and variable head viscosity measurements were made on all samples immediately before centrifuging. Ostwald type viscosimeters were employed, immersed in a constant temperature water bath at 25°C.

Chemical analyses. Nitrogen was determined by a semi-micro Kjeldahl method using mercuric oxide and sodium sulfate. Casein was precipitated with 1 + 9 acetic acid and, after holding the samples for 15 minutes, sodium acetate buffer was added, giving a final pH of 4.6-4.8 (cf. ref. 5). Sometimes with sodium-tetrametaphosphate-treated milk, difficulty in precipitating the casein was encountered, a factor which should be borne in mind in evaluating the analytical results. Casein once precipitated was redispersed in alkali, reprecipitated, and this procedure repeated until the supernatant liquids gave no test for phosphates. Nitrogen and phosphorus were determined on the final wet precipitates.

Total phosphorus was determined by the volumetric method described in A.O.A.C. (1) except that alkaline solutions of the casein samples in platinum crucibles were neutralized with HNO_3 and then made alkaline to phenolphthalein with solid Na_2CO_3 before evaporating on the steam bath, the samples being then ashed at 550°C..

Results and calculations

Sedimentation patterns. *Figure* 1 is a set of sedimentation pictures for a sodium-tetrametaphosphate-treated skim milk prepared

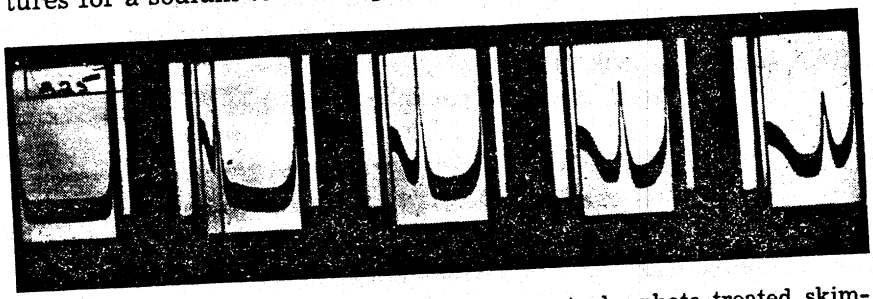

Figure 1. — Sedimentation of sodium-tetrametaphosphate-treated skim-milk at pH 6.69; 50,740 r.p.m., average centrifugal force 186,000 times gravity, intervals 32 minutes. For the main component s_{20}, η_{H_2O}, $x_0 = 9.8 \times 10^{-13}$

as described at pH 6.69. *Figure* 2 is a similar set for a sodiumtetrametaphosphate-treated suspension of casein colloids from the same milk. The casein colloid was separated in a large air-driven bowl rotor, washed twice with distilled water by resuspending and recentrifuging, diluted with water to make a synthetic milk and

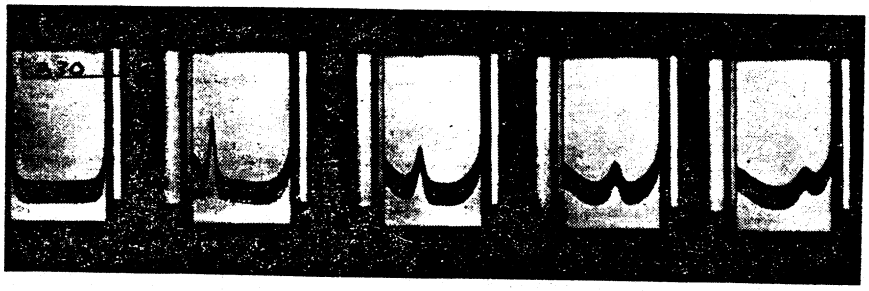

Figure 2. — Sedimentation of a sodium-tetrametaphosphate-treated suspension of washed casein colloids at pH 6.69; 50,740 r.p.m., average centrifugal force 186,000 times gravity, intervals 32 minutes. For the main component, $s_{20^\circ,\eta H_2O,\, x_0} = 9.7 \times 10^{-13}$

this then mixed with buffer at pH 6.69 and treated with sodium tetrametaphosphate by the usual procedure. In both sets of pictures a rudimentary, fast-moving peak is seen to the right of the main component, as well as a composite of several slower-moving peaks to the left of the main peak. Untreated skim milk gives broad and diffuse patterns in the Spinco ultracentrifuge, and the colloids are sedimented in a very few minutes at the centrifugal speed used here. *Figure* 3 is a tracing of a sedimentation pattern obtained at pH 6.54. In this figure the existence of one minor peak at the right of the main peak is clearly indicated; and from the change in slope at the base of the main peak, a second minor component is inferred.

In figure 3 the vertical displacements have been corrected for radial dilution. Taking the sedarate areas as being proportional to quantities of material, for this particular pattern component I represents 42%, component III, 53%, component IV, 2%, and component V. 3% of the total protein. In acid solutions existence of another minor component, II, just to the left of the main component was sometimes indicated.

Graphic analyses, such as indicated, were made of patterns obtained over a wide pH range of treated skim milks. Some of the results are summarized in Table 1. In this table it will be noted that the numbers in columns 2, 3,. 4, and 5 add up to 100. It is

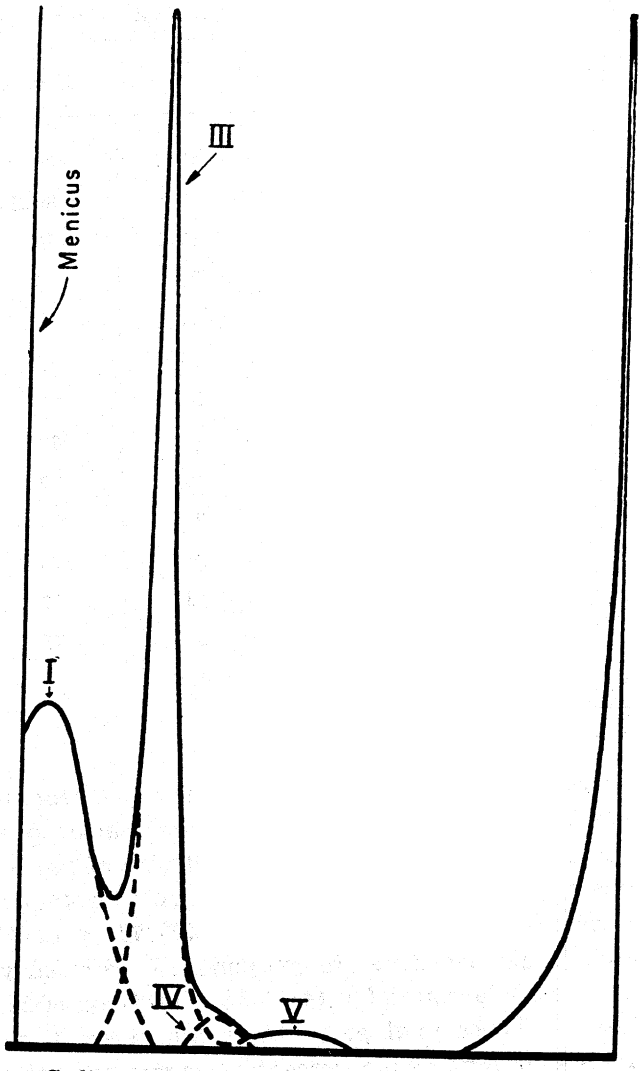

Figure 3. — **Sedimentation pattern derived from a tracing by correcting the vertical displacements for radial dilution, for a sodium-tetrametaphosphate-treated skim milk at pH 6.54; 50,740 r.p.m.; total equivalent centrifuging time, 53 minutes.**

TABLE I. — PROPORTIONS OF COMPONENTS AND DISTRIBUTION OF CASEIN NITROGEN IN SODIUM TETRAMETAPHOSPHATE TREATED SKIM MILKS, BASED ON SEDIMENTATION PATTERNS

pH	Relative Areas Under Peaks				Average Original Serum Protein	% of Original Casein Nitrogen	
	Component I	Component III	Component IV	Component V		as I	as III, IV, V
5.23	38	41[a]	13	8[b]	(23)	19.5	80.5
5.30	38	56	3	3	»	19.5	80.5
5.43	38	57	2	3	»	19.5	80.5
5.76	39	55	2	4	»	21	79
6.33	39	54	3	4	»	21	79
6.54	42	53	2	3	»	25	75
6.64	41	55	3	1	»	23	77
6.75	41	54	3	2	»	23	77
7.04	41	54	3	2	»	23	77
7.51	42	53	3	1	»	25	75
7.83	42	54	2	2	»	25	75
8.47	41	54[c]	3	2	»	23	77
9.20	42	53[c]	5	—	»	25	75

(a) Includes about 5 parts Component II.
(b) Includes a small amount of a larger component.
(c) Peak becoming diffuse.

assumed that the graphic analysis accounts for the total protein. Of the total protein in an average milk sample, 23% is serum protein and 77% is casein. Therefore, taking the first row of figures in Table 1 for illustration, if all of the original serum protein is assumed to be present in component I, then (38-23)/77 or 19.5% of the original casein is also contained in component I. Similarly, components III, IV, and V account for $(41 + 13 + 8)/77$ or 80.5% of the original casein. These final percentages are given in columns 7 and 8. Although appreciable percentage errors are possible in the assigned proportions of the minor components IV and V, the error in calculation of the proportions of component III is small. The various sets of figures are comparable because the

total concentrations of proteins were in all cases approximatcly the same. The figures of Table 1 show that in the normal pH range about 77% of the original casein could be contained under the dominant peak, component III, and faster peaks to the right, and that about 23% of the original casein must be contained in slower moving components.

Sedimentation constants, viscosity and pH. The observed time-interval sedimentation constants were corrected first to 20°C by multiplying by the ratios of the viscosities of water at the calculated interval rotor temperatures and 20°C. In *figure* 4 such interval $S_{OBS.,\,20}$ values calculated from Figure 1 for the main component are plotted against the squares of the interval average distances from the center of rotation. The slope of this plot is the reverse of that usually obtained for proteins. It is typical, however, for all the sodium-tetrametaphosphate-treated milks that were ultracentrifuged. To avoid implications as to the interpretation of this negative slope, the plots were all extrapolated to zero

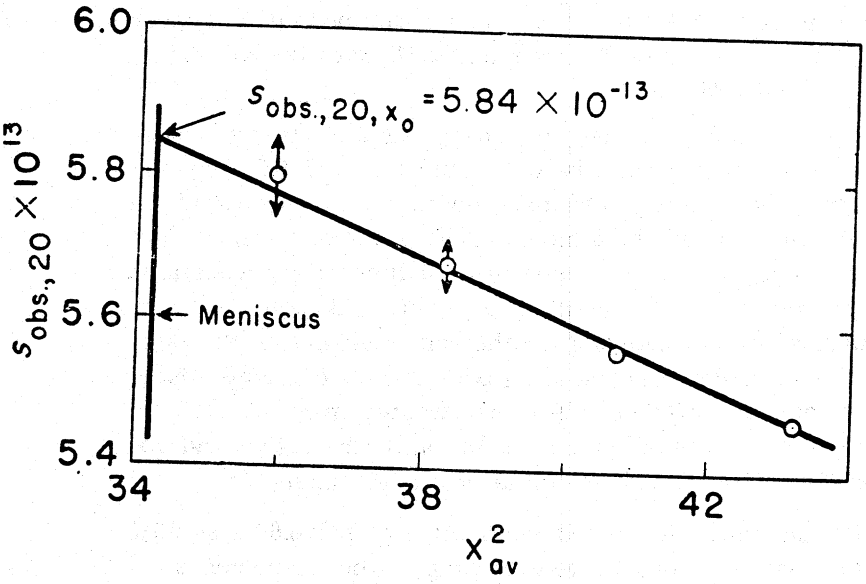

Figure 4. — Relationship of observed interval sedimentation constants at 20° to the squares of the interval average distances of the dominant schlieren peak from the center of rotation, showing the method of extrapolating to zero fall distance. Data taken from Figure 1

fall distance, i. e., to the meniscus. These extrapolated sedimentation constants are assumed to be descriptive of the undisturbed system, and are designated by the symbol $S_{OBS.,\,20,\,x_0}$

The $S_{OBS.,\,20,\,x_0}$ values were further corrected for solution viscosity by multiplying by the ratios of the solution viscosities to the viscosity of water. These final sedimentation constants are designated by the symbol $S_{20,\,\eta\,H_2O,x_0}$ No correction for buoyancy is included because apparent specific volumes were not determined. The $S_{20,\,\eta\,H_2O,x_0}$ values calculated from figures 1 and 2 for the solutions at pH 6.69 prepared from skim milk and washed colloids are for the main component, 9.8×10^{-13} and 9.7×10^{13}, respectively.

Figure 5 shows the change of $S_{20,\,\eta\,H_2O,x_0}$ values at about 1.5% casein for the main component (III) with pH. The attendant changes in solution viscosity also are indicated on this plot. Precipitation begins below about pH 5.5; structural viscosity becomes apparent and increases above pH 7.0; and there is visible gelation above pH 8.4. The pH range 6.5 to 7.8 might be designated as the pH stability range, at least vith respect to the sedimentation constant. The sedimentation constants for component V appear be in constant ratio to those for component III over the pH range. This ratio is about 1.85 to 1.

Effects of dilution. Sodium-tetrametaphosphate-treated skim milk at pH 6.67 diluted serially with buffer at pH 6.67 without further addition of sodium tetrametaphosphate, showed rapid diminution of the main peak, component III, relative to the serum proteins. Extrapolation of the measured sedimentation constants to zero concentration was possible, however, and gave $s^o_{20} = 19 \times 10^{-13}$, approximately. Dilution of the same solution with water led to similar breakdown of the main peak and its complete disappearance at extreme dilution. Another treated milk, at pH 5.53, showed no obvious breakdown on dilution with water, and for this series the extrapolated s^o_{20} value was again about 19×10^{-13}

Molecular weights. A treated skim milk at pH 6.67 was 50% depleted of casein nitrogen by centrifuging in the air-driven bowl rotor with an annular partition. This solution was then centrifuged in the Spinco optical cell for five days at 11,192 r. p. m. From pictures obtained in the early stages of the centrifugation the constants, $S_{OBS..\,20} = 5{,}3 \times 10^{-13}$, and $D_{OBS..\,20} = 1.13 \times 10^{-7}$ were

calculated for component III. The diffusion constant was calculated by the method described by Pedersen (6) assigning all of the boundary spreading to diffusion of component III. Assuming an apparent specific volume of 0.731, the value for casein (4), these figures give for the sedimentation molecular weight, Ms, 450,000

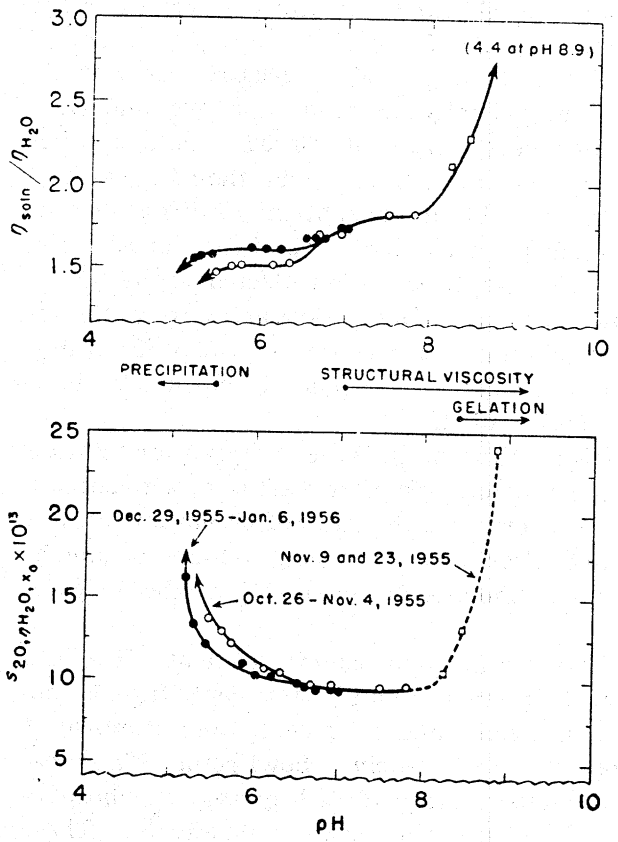

Figure 5. — Relationships of sedimentation constant, viscosity, and state of aggregation to pH, for sodium-tetrametaphosphate-treated skim milks.

The last patterns obtained, on the fifth day, could be partially resolved giving an approximately linear log concentration-$x^2_{av.}$ plot for the top three quarters of the column, indicating an average equilibrium molecular weight of the order of 50,000. The concentrations given by the extrapolation of this plot were substracted from the observed total concentrations in the bottom one quarter

of the column. These differences gave a second log concentration-$x^2_{av.}$ plot which was linear within the errors of measurement. This latter plot was very steep and gave an equilibrium molecular weight of 400,000, approximately. In the calculations the apparent specific volume 0.731 was assumed.

A treated suspension of washed colloids at pH 6.69 was similarly centrifuged for six days at 6,944 r.p.m. In this case for the main component the constants, $S_{OBS.,\,20} = 6.6 \times 10^{-13}$ and $D_{OBS.,\,20} = 1.47 \times \times 10^{-7}$ were found. These values give $M_s = 440{,}000$. Here again, the final pattern could be partially resolved, as above, and gave an equilibrium molecular weight of about 520,000. The resolution in this latter experiment was more satisfactory than in the first.

Appreciable errors are possible in calculations of diffusion constants from sedimentation patterns, and in resolution of complex equilibrium patterns. The first of these is reflected in the M_s values, the second in the M_e values. Despite possible errors, the results are useful in that they indicate a relatively low order of molecular weight for the proteins of component I, and a very high molecular weight for component III.

Analytical data. Several skim milks were analyzed for total nitrogen and casein nitrogen before and after treatment with sodium tetrametaphosphate. In every case the ratio of casein nitrogen to total nitrogen decreased after treatment with sodium tetrametaphosphate. The decrease amounts to about 11% of the original casein nitrogen.

Casein was precipitated with hydrochloric acid at pH 4.6-4.8 from skim milk, from the same skim milk after centrifugal removal of 33% of the casein colloid, and after centrifugal removal of 51% of the casein colloid in the air-driven bowl rotor. These caseins were thoroughly washed and analyzed, giving the phosphorus:nitrogen ratios 0.056, 0.056, and 0.057, respectively. These ratios are in good agreement with an average ratio 0.0563 based on analyses of centrifugally separated colloids previously reported (3). The same skim milk and the 33% depleted and 51% depleted skim milks were treated with buffer and sodium tetrametaphosphate, at pH 6.69, by the usual procedure. The caseins precipitated from these treated solutions were washed and analyzed as described. For these caseins the phosphorus:nitrogen ratios found were 0.077, 0.074, and 0.077, respectively.

Sodium-tetrametaphosphate-treated skim milk, at pH 6.78, was centrifuged in the Spinco separation cell until the main peak, component III, was observed to pass completely through the filter paper barrier. The centrifuge was stopped. Analysis of the liquid removed from above the barrier gave 0.016% casein nitrogen and 0.096% total nitrogen as compared with 0.229 casein nitrogen and 0.0324% total nitrogen before centrifuging. In this case component I above the barrier could be partially resolved, giving a peak midway of the pattern having a sedimentation constant, S_{20,η_{H_2O},x_0} of about 2.6×10^{-13}. Since at this pH, for component III, $S_{20,\eta_{H_2O},x_0} = 9.7 \times 10^{-13}$, the 0.016% casein nitrogen above the barrier represents about 3/4 of that originally present as component I. The amount originally present was, therefore, about 0.021%. Thus [(0.229-0.021)/0.229] $\times$ 100 or 90.8% of the casein nitrogen in the treated skim milk is accounted for as components III, IV, and V. If sodium tetrametaphosphate had not been added the original 0.324% total nitrogen would have represented 0.250% casein nitrogen, by proportion from the analysis of the original skim milk. Thus (0.208/0.250) $\times$ 100 or 83% of the original casein nitrogen present before treatment is accounted for as components III, IV, and V. This figure compares favorably with the corresponding figures based on sedimentation patterns which are given in the last colum of Table I. Parallel experiments with the air-driven bowl rotor confirmed this quantitative result, and showed also no detectable separation of components sedimenting ahead of component V in the normal pH range. These might have been overlooked by the optical technique.

Comparison with α-casein and with mixtures of α-, β-, and γ-caseins. Electrophoretically pure freeze-dried α-casein was dissolved in phosphate buffer at pH 7.5 and ionic strength 0.10. The total protein concentration was 1.5%. A sharp ultracentrifugal peak was obtained giving $S_{20,\eta_{H_2O},x_0} = 4.4 \times 10^{-13}$. On adding sodium tetrametaphosphate to this solution, and to other α-casein solutions throughout the pH range investigated, precipitation occured. A mixture of α-, β-, and γ-caseins prepared by dispersing an unfractionated washed urea precipitate of these proteins from milk was diluted with phosphate buffer at pH 7.5 to a concentration of 1.7%. It found that sodium tetrametaphosphate could be

added to this solution without precipitation and that the pH dropped to 6.9. The sedimentation constant S_{20,η_{H_2O},x_0} for the dominant component in this mixture was 4.4×10^{-13} before adding sodium tetrametaphosphate and 7.2×10^{-13} afterward. Comparison of the picture shows absence of serum proteins before treatment, but the presence of a considerable amount of slow-moving material, giving a pattern similar to component I, after treatment. This new component, derived from the α -, β -, γ - casein mixture, represents 25 to 30% of the total protein.

ABSTRACT

The casein colloids in skim milk can be reduced in size and translucent liquids obtained by adding sodium tetrametaphosphate. Such treated skim milks in buffers over a wide pH range were studied in the ultracentrifuge. One dominant centrifugally homogeneous component is always found. The sedimentation velocity of this component is constant in the normal pH range and increases on both the acid and alkaline sides, leading in the first case to precipitation, in the second to gelation. This dominant component has a molecular weight, in the normal pH range, of about 500.000. The phosphorus content of casein precipitated from sodium tetrametaphosphate-treated skim milk is about 40% greater than that of normal casein and at the same time a part of the original casein is non-acid precipitable. The dominant component plus small amounts of components of similar size account for about 80% of the original casein. The remainder of the original casein which is only in part acid-precipitable sediments with the serum proteins. The new high molecular weight substance is not produced by sodium tetrametaphosphate treatment of fresh laboratory α - casein alone, but a substance exhibiting similar ultracentrifugal behavior is produced by such treatment of freshly precipitated mixtures of α -, β -, and γ - caseins.

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SUMMARY

The system skim milk-buffer-sodium tetrametaphosphate contains one dominant centrifugally homogeneous component (III) of molecular weight about 500,000, which with small amounts of one or two other components (II, IV, and V), can account for roughly 80% of the casein originally present in the skim milk. The remaining 20% of the original casein sediments with the serum proteins and of this about one half appears to be no longer acid precipitable. The sedimentation constant of the dominant component III is constant between pH 6.5 and 7.8, approximately, and increases on both the acid and alkaline sides, leading in the first case to precipitation and in the second to gelation.

The phosphorus content of casein precipitated from sodium-tetrametaphosphate-treated milk is about 40% greater than that of normal casein. This analytical result plus the fact that pure α-casein is precipitated from buffer solution by sodium tetrametaphosphate, shows that the new substance is not α-casein, nor an aggregate of α-casein. The phosphorus analyses also show that the substance is not a simple aggregate of α-, β-, and γ-caseins. That at least one of these other caseins is involved, however, is shown by the resistance of a mixture of all three to precipitation by sodium tetrametaphosphate, and by transformations similar to those observed with skim milk as indicated by sedimentation velocity measurements.

RESUME

LES EFFETS DU TETRAMETAPHOSPHATE SODIUM SUR LA CASEINE A L'ETAT COLLOIDAL DANS LE LAIT

Le système: lait écrémé-solution régulatrice-tétramétaphosphate sodium contient un composé homogène dominant centrifugement

(III) d'un poids moléculaire de 500,000 à peu prés, qui avec de petites quantités d'un et de deux autres composés (II, IV et V) peut expliquer, plus ou moins, le 80% de la caséine présente à l'origine dans le lait écrémé. Le 20% restant de sédiments originaux de caséine avec les séroprotéines et presque la moitié de ceux-ci paraissent ne plus être précipitables dans l'acide. La sédimentation constante du composé dominant III demeure constante entre pH 6,5 et 7,8, approximativement et augmente aussi bien dans l'acidité et dans l'alcalinité, menant dans le premier cas à une précipitation et dans le second cas à la gelifisation.

La teneur en phosphore de la caséine précipitée à partir du lait traité avec du tétramétaphosphate de sodium est de 40% supérieure à peu près à celle de la caséine normale. Les résultats de ces analyses, plus le fait que la caséine α pure est précipitée de la solution régulatrice par le tétramétaphosphate de sodium montre que la nouvelle substance n'est pas la caseine α ni un agrégat de caséine α.

Les analyses de phosphore montrent aussi que la substance n'est pas un simple agrégat de caséine α, β, et γ.

Qu'au moins une de ces autres caséines est comprise, est prouvé du reste par la résistance du mélange de toutes les trois à la précipitation par le tétramétaphosphate de sodium et par des transformations similaires à celles observées avec le lait écrémé comme l'indique le mesurage de la rapidité de sédimentation.

ZUSAMMENFASSUNG

WIRKUNG VON NATRIUMTETRAMETAPHOSPHAT AUF DAS KASEIN-KOLLOID IN DER MILCH

Das System: Magermilch-Puffer-Natriumtetrametaphosphat enthält eine zentrifugal-homogene Komponente (III) mit einem Molekulargewicht von etwa 500.000, die zusammen mit geringen Mengen von ein bis zwei anderen Bestandteilen (II, IV und V) rund 80% des ursprünglich in der Magermilch vorhandenen Kaseins ausmachen kann. Die übrigen 20% des ursprünglichen Kaseins setzen sich zusammen mit den Serumproteinen ab, und etwa die Hälfte davon erscheint durch Säuren nicht mehr ausfällbar. Die Sedimentation der vorherrschenden Komponente III ist unverän-

derlich zwischen etwa pH 6,5 und 7,8. Sie nimmt sowohl nach der sauren wie nach der alkalischen Seite hin zu und führt im ersteren Fall zur Ausfällung, in letzteren zur Gel-Bildung.

Der Phosphorgehalt des Kaseinniederschlags aus Natriumtetrametaphosphat-behandelter Milch ist ungefähr 40% höher als derjenige des normalen Kaseins. Dieses Analysenergebnis in Verbindung mit der Tatsache, dass reines α-Kasein aus Pufferlösung durch Natriumtetrametaphosphat ausgefällt wird, zeigt, dass es sich bei der neuen Substanz nicht um α - Kasein noch um ein Aggregat von α -, β - und γ - Kasein handelt. Dass aber mindestens eins dieser anderen Kaseine beteiligt ist, geht hervor aus der Resistenz, die eine Mischung aller drei der Fällung durch Natriumtetrametaphosphat entgegensetzt, und aus Umformungen ähnlich denen, wie man sie bei Magermilch beobachtet und Messung der Geschwindigkeit der Sedimentation sie anzeigt.

RESUMEN

LOS EFECTOS DEL TETRAMETAFOSFATO DE SODIO EN LA CASEINA EN ESTADO COLOIDAL EN LA LECHE

El sistema leche descremade-disolución reguladora-tetrametafosfato de sodio contiene un componente dominante centrífugamente homogéneo (III) de un peso molecular aproximado de 500,000, el cual, con pequeñas cantidades de otro u otros dos componentes (II, IV y V), puede explicar el 80% más o menos de la caseína presente originalmente en la leche descremada. El 20% restante de los sedimentos originales de la caseína con las seroproteínas y de éstas aproximadamente la mitad, no son ya, al parecer, precipitables en ácido. La sedimentación constante del componente dominante III es constante entre pH 6,5 y 7,8, aproximadamente, y aumenta tanto en el extremo ácido como en el alcalino, llevando en el primer caso a la precipitación y en el segundo a la gelación.

El contenido de fósforo de la caseína precipitada a partir de la leche tratada con tetrametafosfato de sodio, es superior en un 40% al de la caseína normal. El resultado analítico, además del hecho de que la α - caseína pura se precipita de la solución reguladora por el tetrametafosfato de sodio, demuestra que la nueva substancia no es α - caseína, ni un conjunto de α - caseína. Los análisis de fósforo

demuestran también que la substancia no es un simple conjunto de α-, β-, y γ-caseínas. Sin embargo, el que se halla implicada por lo menos una de estas otras caseínas, lo demuestra la resistencia de una mezcla de las tres a la precipitación por tetrametafosfato de sodio, y por transformaciones análogas a los observadas con la leche descremada, como lo indican las medidas de velocidad de sedimentación.

RIASSUNTO

GLI EFFETTI DEL TETRA-METAFOSFATO DI SODIO SUI COLLOIDI CASEINICI NEL LATTE

Il sistema: latte scremato — soluzione tampone — tetrametafosfato di sodio — contiene un componente principale centrifugalmente omogeneo (III) a peso molecolare di circa 500.000 che insieme con piccole quantità di uno o due altri componenti (II, IV e V) rappresenta circa l'80% della caseina inizialmente presente nel latte scremato. Il rimanente 20% della caseina originale forma un sedimento e sembra che circa la metà di tale sedimento non sia più precipitabile dagli acidi.

La costante di sedimentazione del costituente principale III rimane invariata tra circa pH 6,5 e 7,8 e aumenta tanto la reazione acida quanto quella alcalina, portando nel primo caso alla precipitazione e nel secondo alla gelificazione.

Il contenuto di fosforo nella caseina precipitata dal latte trattato col tetrametafosfato di sodio è di circa 40% superiore a quello della caseina normale. Questo risultato analitico e il fatto che l'α-caseina pura è precipitata nella soluzione tampone per azione del tetrametafosfato di sodio, dimostrano che la nuova sostanza non è un'α-caseina nè un composto di essa.

Le analisi di fosforo dimostrano pure che tale sostanzà non è semplice combinazione di α-, β-, γ-, caseine. Però la presenza di almeno una di tali caseine è dimostrata dalla resistenza della miscela di tutte e tre le proteine alla precipitazione per azione del tetrametafosfato di sodio e per trasformazioni analoghe a quelle csservate nel latte scremato come indicato dalla velocità di sedimentazione.

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